Fermentation Test for Total Coliform (9221B), Fecal Coliform (9221E) and *E. coli* (9221F) SM 9221 – 2006

ADDITIONAL QC REQUIREMENTS FOR THIS METHOD: Certified or Accredited laboratories using this method are assessed to applicable requirements of SM 9020, 9031, 9040, 9050 & 9060.

Facility Name:_____ LAB ID:_____

Assessor Name:_____ Analyst Name:_____ Inspection Date:_____

Records Examined: SOP Number/Revision/Date:_____ Analyst:_____

Sample ID:_____ Date of Sample Preparation:_____ Date of Analysis:_____

Relevant Aspect of Standards	Method Reference	Y	N	N/A	Comments
1) For nonpotable water, are samples analyzed within 8 hours of collection?	**40 CFR 136.3 Table 1l**				
2) For drinking water, are samples analyzed within 30 hours of collection?	**40 CFR 141.21(f)(3) Footnote 2, EPA 815-R-05-004 Jan. 2005 Ch V Sec. 6.44**				
3) For surface water, are total coliform and fecal coliform samples analyzed within 8 hours of collection?	**40 CFR 141.74(a)(1) Footnote 2**				
4) Are samples preserved at <10°C?	**40 CFR 136.3 Table 1l**				
Presumptive Phase (Lauryl Tryptose Broth) *[This section applies to ALL methods EXCEPT 9221E.2. A-1 Medium – See page 4]*					
5) Is lauryl tryptose broth (LTB) used?	9221 B 2				
6) If LTB was refrigerated after sterilization, was it incubated at 20°C overnight prior to use so that tubes showing growth and/or bubbles could be discarded? *NOTE: Incubation of a sterility blank of LTB along with test batch satisfies this requirement.*	9221 B 2				
7) Before sterilization, is LTB dispensed into fermentation tubes, covering the inverted vial at least one-half to two-thirds after sterilization? *[Alternatively, omit the inverted vial and add 0.01 g/L bromocresol purple to LTB to determine acid production (yellow color), as indicator of a positive result.]*	9221B.2.a				
8) Is LTB prepared according to Table 9221:I?	9221B.2.a				

Notes/Comments

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Facility Name:_____ LAB ID:_____

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Records Examined: SOP Number/Revision/Date:_____ Analyst:_____

Sample ID:_____ Date of Sample Preparation:_____ Date of Analysis:_____

9)	TABLE 9221:I. PREPARATION OF LAURYL TRYPTOSE BROTH Inoculum *mL* / Amount of Medium in Tube *mL* / Volume of Medium + Inoculum *mL* / Dehydrated Lauryl Tryptose Broth Required *g/L* 1 / 10 or more / 11 or more / 35.6 10 / 10 / 20 / 71.2 10 / 20 / 30 / 53.4 20 / 10 / 30 / 106.8 100 / 50 / 150 / 106.8 100 / 35 / 135 / 137.1 100 / 20 / 120 / 213.6					
10)	After sterilization, are inverted vials free of air bubbles?	9221B.2.a				
11)	After sterilization, is medium pH 6.8 ± 0.2? ("Should")	9221B.2.a				
12)	NONPOTABLE WATER: Are five tubes per dilution (of 10, 1, 0.1 mL, etc.) added to LTB tubes?	9221B.2.b.1				
13)	DRINKING WATER: Are five 20 mL portions, ten 10 mL portions, or a single 100 mL portion added to LTB tubes or bottles?	9221B.2.b.1				
14)	Are samples and dilutions shaken vigorously about 25 times? Are test portions in the medium mixed by gentle agitation?	9221B.2.b.1				
15)	Were inoculated tubes or bottles incubated at 35±0.5°C and examined for growth and gas OR acidic reaction (yellow color) after 24±2 hours?	9221B.2.b.2				
16)	If no gas or acidic reactions were evident after 24±2 hours, were inoculated tubes or bottles reincubated and reexamined after 48 ± 3 hours?	9221B.2.b.2				

Notes/Comments

Fermentation Test for Total Coliform (9221B), Fecal Coliform (9221E) and *E. coli* (9221F) SM 9221 – 2006

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Relevant Aspect of Standards	Method Reference	Y	N	N/A	Comments
17) Does production of gas OR acidic reaction (yellow color) in the tubes or bottles within 48 ± 3 h constitute a positive presumptive test?	9221B.2.c				
18) Does the absence of gas formation OR acidic reaction at the end of 48±3 h of incubation constitute a negative test?	9221B.2.c				
19) DRINKING WATER: Are presumptive tubes or bottles demonstrating growth without a positive gas or acidic reaction submitted to the confirmed phase (9221B.3)?	9221B.2.c				
20) NPW & SOLIDS: Are positive presumptive tubes submitted to the confirmed phase (9221B.3) for fecal coliform OR *E. coli*? (*For waters or wastewaters known to be consistently polluted only the highest dilution need be moved to confirmed.*)	9221B.2.c, B.3.c				
21) DRINKING WATER: Are positive presumptive tubes or bottles submitted to the confirmed phase (9221B.3) for total coliform AND fecal coliform or *E. coli*?	9221B.2.c & 9221A.1				
NOTE – Checklist does not follow the order of SM 9221. SM9221E Fecal Coliform confirmation is on page 3, SM9221F *E. coli* confirmation is on page 5, and SM 9221B Total Coliform is on page 6.					
SM 9221E 1 Fecal Coliform Confirmation Test (EC Medium)					
22) Before sterilization, is EC medium dispensed into fermentation tubes, covering the inverted vial at least one-half to two-thirds after sterilization?	9221E.1.a				
23) Is pH of EC medium 6.9 ± 0.2 after sterilization? *("Should")*	9221E.1.a				
24) Are inverted vials free of air bubbles?	9221E.1.a				
25) Are positive presumptive tubes gently shaken and then transferred to EC broth?	9221E.1.b.1				
26) Are EC broth tubes placed in water bath	9221E 1.b.2				

Notes/Comments

Fermentation Test for Total Coliform (9221B), Fecal Coliform (9221E) and *E. coli* (9221F) SM 9221 – 2006

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within 30 minutes of inoculation?					
27) Is sufficient water depth maintained so tubes are immersed to the upper level of the medium?	9221E 1.b.2				
28) Are inoculated EC tubes incubated in a water bath at 44.5 ± 0.2°C for 24 ± 2 hours?	9221E.1.b.2				
29) Are EC broth tubes showing growth and gas production considered positive for fecal coliform?	9221E.1.c				
30) Is failure to produce gas (with little or no growth) recorded as a negative reaction?	9221E.1.c				
31) If multiple tubes are used, is the Most Probable Number (MPN/100 mL of fecal coliforms calculated from the number of positive EC broth tubes as described in 9221C on pg 8 of checklist?	9221E.1.c, 9221 C 2				
32) Drinking Water Presence/Absence (P/A): Are results reported as presence/absence if using only one EC Medium tube subcultured from a single presumptive bottle?	9221E.1.c				
SM 9221E 1 Fecal Coliform Direct Test (A-1 Medium)					
33) Is dehydrated medium heated in reagent grade water to dissolve solid ingredients?	9221E.2.a				
34) Is polyethylene glycol *p*-isooctylphenyl ether added after medium is dissolved?	9221E.2.a				
35) Is medium pH adjusted to pH 6.9 ± 0.1 before sterilization?	9221E.2.a				
36) Is double-strength medium prepared for analyzing 10 mL sample volumes?	9221E.2.a				
37) Before sterilization, is sufficient medium dispensed into fermentation tubes to cover the inverted vial at least one-half to two-thirds after sterilization?	9221E.2.a				
38) Is medium sterilized at 121°C for 10 minutes?	9221E.2.a				

Notes/Comments

Fermentation Test for Total Coliform (9221B), Fecal Coliform (9221E) and *E. coli* (9221F) SM 9221 – 2006

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39) Are inverted vials are free of air bubbles?	9221E.2.a				
40) Is medium stored in the dark at room temperature for no more than 7 days? *NOTE: Ignore formation of precipitate during storage.*	9221E.2.a				
41) Immediately before inoculating tubes, are samples and dilutions shaken vigorously about 25 times?	9221E.2.b, 9221B.2.b.1				
42) Are the appropriate portions of sample or dilution added to broth tubes? Are five tubes per dilution (of 10, 1, 0.1 mL, etc.) used?	9221 E 2 b., 9221B.2.b.1				
43) Are broth tubes inoculated and then incubated for 3 hours at 35 ± 0.5°C, followed by 21 ± 2 hours in a water bath at 44.5 ± 0.2°C?	9221E 2.b				
44) Are tubes considered positive for fecal coliform only if gas production occurred?	9221E 2.c				
45) Is MPN/100 mL calculated from the number of positive A-1 tubes by referring to 9221 C on page 8 of checklist?	9221E 2 c, 9221C 2				
SM 9221F *Escherichia coli* Confirmation Test (EC-MUG Medium)					
46) Was EC-MUG medium dispensed into tubes that did not fluoresce under long-wavelength (366 nm) UV light so as not to interfere with later interpretation? *NOTE: Inverted tube is not required.*	9221F 1.a				
47) Is pH of EC-MUG medium within 6.9 ± 0.2 after sterilization? (“Should”)	9221F 1				
48) Are positive presumptive tubes or bottles gently shaken and then transferred to EC broth?	9221F.1.b.1				
49) Are inoculated EC-MUG tubes incubated in a water bath at 44.5 ± 0.2°C for 24 ± 2 hours?	9221F.1.b.2				

Notes/Comments

Fermentation Test for Total Coliform (9221B), Fecal Coliform (9221E) and *E. coli* (9221F) SM 9221 – 2006

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50) Were inoculated tubes placed in incubator or water bath within 30 minutes after inoculation?	9221F.1.b.2				
51) Does water bath have sufficient water depth to immerse tubes to the upper level of the medium?	9221F.1.b.2				
52) Are tubes exhibiting growth examined for fluorescence under a 6W, 365-366 nm UV light after incubation?	9221 F.1.c				
53) Is the presence of bright-blue fluorescence considered a positive result for *E. coli*?	9221 F.1.c				
54) Is growth in the absence of bright blue fluorescence considered a negative result?	9221 F.1.c				
55) If multiple tubes are used, is the MPN/100 mL for *E. coli* calculated from the number of positive EC-MUG broth tubes as described in 9221C on page 8 of checklist?	9221 F.1.c				
56) DRINKING WATER: When using only one tube, or subculturing from a single presumptive bottle, is the presence or absence of *E. coli* reported?	9221 F.1.c				
57) Are a positive control consisting of a known *E. coli* (MUG-positive) culture, a negative control consisting of a thermotolerant *Klebsiella pneumoniae* (MUG-negative) culture, and an uninoculated medium control included in the assay?	9221 F.1.c				
SM 9221B 3 Total Coliform Confirmation Test in BGLB Broth					
58) Are brilliant green lactose bile broth (BGLBB) fermentation tubes used for the confirmed phase?	9221 B.3.a				
59) Before sterilization, is BGLBB dispensed into fermentation tubes, covering the inverted vial at least one-half to two-thirds after sterilization?	9221 B.3.a				

Notes/Comments

Fermentation Test for Total Coliform (9221B), Fecal Coliform (9221E) and *E. coli* (9221F) SM 9221 – 2006

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60) Are inverted vials free of air bubbles?	9221 B.3.a				
61) Is medium pH 7.2 ± 0.2 after sterilization? ("Should")	9221 B.3.a				
62) Are positive presumptive tubes or bottles gently shaken or rotated to resuspend the organisms?	9221 B.3.b				
63) Are presumptive tubes gently shaken and then transferred to BGLBB?Is this process repeated for all other positive presumptive tubes?	9221 B.3.b				
64) Are inoculated BGLBB tubes incubated at 35 ± 0.5°C?	9221 B.3.b				
65) Is the formation of gas in any amount in the inverted vial of the BGLBB fermentation tube at any time within 48 ± 3 h considered a positive confirmed phase?	9221 B.3.b				
66) Is the total coliform density estimated by calculating the MPN/100 mL from the number of positive BGLBB tubes as described in 9221C on page 8 of checklist?	9221 B.3.b				
67) NONPOTABLE WATER: Is the Completed Test performed as described in 9020B 10 a 1) b)?*(If no positive sample occurs within a quarter, then a QC check must be performed using a known positive sample.)*	9221 B.4				
68) Is the Completed Test one of the following? a) Simultaneous inoculation into BGLBB for total coliform and EC broth for fecal coliform or EC MUG for E. coli b) Positive results from incubation in EC and/or EC-MUG broths at 44.5±0.2°C c) Inoculation into LES Endo agar or MacConkey agar plate from positive BGLBB tube and incubate at 35±0.5°C	9221 B.4				
9221 C. Estimation of Bacterial Density – See Page 8					

Notes/Comments

Fermentation Test for Total Coliform (9221B), Fecal Coliform (9221E) and *E. coli* (9221F) SM 9221 – 2006

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TABLE 9221:IV. MPN INDEX AND 95% CONFIDENCE LIMITS FOR VARIOUS COMBINATIONS OF POSITIVE RESULTS WHEN FIVE TUBES ARE USED PER DILUTION (10 mL, 1.0 mL, 0.1 mL)*

Combination of Positives	MPN Index/100 mL	Confidence Limits		Combination of Positives	MPN Index/100 mL	Confidence Limits	
		Low	High			Low	High
0-0-0	<1.8	—	6.8	4-0-3	25	9.8	70
0-0-1	1.8	0.090	6.8	4-1-0	17	6.0	40
0-1-0	1.8	0.090	6.9	4-1-1	21	6.8	42
0-1-1	3.6	0.70	10	4-1-2	26	9.8	70
0-2-0	3.7	0.70	10	4-1-3	31	10	70
0-2-1	5.5	1.8	15	4-2-0	22	6.8	50
0-3-0	5.6	1.8	15	4-2-1	26	9.8	70
1-0-0	2.0	0.10	10	4-2-2	32	10	70
1-0-1	4.0	0.70	10	4-2-3	38	14	100
1-0-2	6.0	1.8	15	4-3-0	27	9.9	70
1-1-0	4.0	0.71	12	4-3-1	33	10	70
1-1-1	6.1	1.8	15	4-3-2	39	14	100
1-1-2	8.1	3.4	22	4-4-0	34	14	100
1-2-0	6.1	1.8	15	4-4-1	40	14	100
1-2-1	8.2	3.4	22	4-4-2	47	15	120
1-3-0	8.3	3.4	22	4-5-0	41	14	100
1-3-1	10	3.5	22	4-5-1	48	15	120
1-4-0	10	3.5	22	5-0-0	23	6.8	70
2-0-0	4.5	0.79	15	5-0-1	31	10	70
2-0-1	6.8	1.8	15	5-0-2	43	14	100
2-0-2	9.1	3.4	22	5-0-3	58	22	150
2-1-0	6.8	1.8	17	5-1-0	33	10	100
2-1-1	9.2	3.4	22	5-1-1	46	14	120
2-1-2	12	4.1	26	5-1-2	63	22	150
2-2-0	9.3	3.4	22	5-1-3	84	34	220
2-2-1	12	4.1	26	5-2-0	49	15	150
2-2-2	14	5.9	36	5-2-1	70	22	170
2-3-0	12	4.1	26	5-2-2	94	34	230
2-3-1	14	5.9	36	5-2-3	120	36	250
2-4-0	15	5.9	36	5-2-4	150	58	400
3-0-0	7.8	2.1	22	5-3-0	79	22	220
3-0-1	11	3.5	23	5-3-1	110	34	250
3-0-2	13	5.6	35	5-3-2	140	52	400
3-1-0	11	3.5	26	5-3-3	170	70	400
3-1-1	14	5.6	36	5-3-4	210	70	400
3-1-2	17	6.0	36	5-4-0	130	36	400
3-2-0	14	5.7	36	5-4-1	170	58	400
3-2-1	17	6.8	40	5-4-2	220	70	440
3-2-2	20	6.8	40	5-4-3	280	100	710
3-3-0	17	6.8	40	5-4-4	350	100	710
3-3-1	21	6.8	40	5-4-5	430	150	1100
3-3-2	24	9.8	70	5-5-0	240	70	710
3-4-0	21	6.8	40	5-5-1	350	100	1100
3-4-1	24	9.8	70	5-5-2	540	150	1700
3-5-0	25	9.8	70	5-5-3	920	220	2600
4-0-0	13	4.1	35	5-5-4	1600	400	4600
4-0-1	17	5.9	36	5-5-5	>1600	700	—
4-0-2	21	6.8	40				

* Results to two significant figures.

Notes/Comments